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Rate of Transformation of Atrazine and Bentazone in Water-Saturated Sandy Subsoils

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Abstract: When pesticides leach through the soil to the upper groundwater zone, it is important to know whether further transformation occurs before the pumping wells for drinking water are reached. Atrazine and bentazone were incubated (at 10°C) in five water-saturated sandy subsoils (collected at depths between 1·5 and 3·5 m), simulating the conditions in the field. In three subsoils with comparatively low pH and intermediate to high redox potential, atrazine was transformed gradually, to leave 1·9%, 6·2% and 17·5% of the dose after about five years. In one of these subsoils, hydroxy-atrazine was detected; the amount corresponded to half of the dose of atrazine. In one anaerobic subsoil with high pH, the transformation of atrazine was comparatively fast (half-life about 0·15 year). Another anaerobic subsoil, with similar pH and a somewhat higher redox potential, however, showed hardly any transformation. Sterilization of the first anaerobic subsoil had no effect on the rate of transformation. In the course of about five years, bentazone in the first three subsoils was transformed gradually to leave $<0{\cdot}25\%$, 11% and 25% of the dose. Bentazone transformation in the two subsoils with high pH and low redox potential was very slow, but the presence of oxygen in one of these subsoils speeded up the transformation. © 1998 SCI

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1 INTRODUCTION

Groundwater can be contaminated by the leaching of pesticides, as has been shown in surveys of measurements in shallow and deep groundwater in agricultural areas.[1,2] In many areas, groundwater is used as a source for drinking-water supply. Several catchment areas of suitable groundwater include agricultural fields on which pesticides are used for crop protection. The maximum admissible concentration in the European Union for any pesticide in drinking water[3] is $0{\cdot}1\ \mu g\ dm^{-3}$. In the revised Uniform Principles in Annex VI to Directive 91/414/EEC, this limit also applies to groundwater, provided the toxicological limit is not lower.[4] The leaching of even a very small fraction of a pesticide dosage may mean that this concentration is exceeded.

The transformation of pesticide residue leached from the unsaturated soil zone may continue in the groundwater zone, as was shown in a study[5] of the transformation of the two toxic oxidation products of the insecticide/nematicide aldicarb in four water-saturated subsoils at 10°C. The half-life of aldicarb sulfoxide in three aerobic to slightly anaerobic subsoils at the lowest (most realistic) concentration level ranged from one to three years, while that for aldicarb sulfone ranged from three to eight years. In the fourth, anaerobic, subsoil the half-lives were shorter than 0·1 year. So the rate of pesticide transformation in subsoils may vary greatly, depending on the chemical conditions.

The time for the transport of a residue from the upper groundwater to a nearby pumping well often ranges from several years to a few decades, which provides ample time for further transformation. Pesticide regulations in the Netherlands take account of the possible transformation of a pesticide on its way through the

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groundwater zone over a period of four years. Within that period, the residue entering the upper groundwater must have been transformed to such a degree that the EU guideline value is met.

The herbicides atrazine and bentazone are widely used in crops grown in groundwater catchment areas used for drinking-water supply. As early as the 1980s, atrazine and bentazone were detected[6] in the shallow groundwater of a field with humic sandy soil grown with maize, at concentrations of up to 0·8 μg dm^{-3}. A recent review of atrazine and bentazone measurements in the upper groundwater of agricultural fields in the Netherlands[7] shows that they were detected repeatedly at concentrations above the EU guideline value. The movement of bentazone through the humic sandy soil profile of two fields has been studied[8,9] in the framework of testing a pesticide leaching model under field conditions.

In studies of the transformation of pesticides in aquifer materials, it is important to simulate field conditions as closely as possible, but this has not always been the case. Some researchers[10,11] have used unrealistically high initial atrazine concentrations: 30 mg dm^{-3} and 20 mg dm^{-3}. The high initial concentration seemed to induce the bacterial enzymes to transformation within a few months, whereas no clear transformation was detected[11] at an initial concentration of 200 μg dm^{-3}. Furthermore, it has been found that presence of the solid phase, besides the groundwater, can be essential for the transformation.[11] The addition of a formulated atrazine product to aquifer material, as has been done in some studies,[11] does not seem to be realistic. The temperature of the groundwater zone in temperate regions is usually around 10°C. In spite of this, temperatures of 20°C and 25°C have been used for incubations of atrazine in subsoil material.[10,11]

The present study measured the transformation rates of atrazine and bentazone in water-saturated subsoils with different chemical characteristics. Subsoil conditions were characterized by their pH and redox potential. Most of the five subsoils were collected in areas which are vulnerable to the leaching of pesticides and where the groundwater is suitable as a source of drinking water.

2 MATERIALS AND METHODS

2.1 Locations and subsoil collection

The subsoils used in this study were collected at five locations with fresh groundwater in the Netherlands: Borgerswold, Papenvoort, Vierlingsbeek, Genderen and Wassenaar. Some of these locations are situated in the catchment area of a groundwater pumping station. Borgerswold and Papenvoort are located in the north-east of the Netherlands, in an area with intensive arable farming of e.g. starch potatoes. The soils developed in fluvioglacial and aeolic sandy deposits. The soil of the field in Borgerswold is a reclaimed peat soil with a sandy subsoil. The groundwater table was at a depth of 1·1 m. The soil of the field in Papenvoort is a humic sand podsol with a sandy subsoil. The groundwater table was at a depth of 0·7 m. The Vierlingsbeek location, in the south-east of the Netherlands, is typical for maize cropping. The soil, developed in fluviatile and aeolic deposits, is a humic sand podsol with a sandy subsoil. The groundwater table was at a depth of 2 m.

The soil at the Genderen location developed in a loam deposit of the river Rhine. The subsoil is fluviatile sand, containing some remains of wood and peat. In both years of subsoil collection (1988 and 1989; sites 30 m apart), the groundwater table was at a depth of 1·1 m. The two fields in Wassenaar (about 4 km apart), located in the dune area along the sea shore, were formed by levelling the lower dunes on the inland side. They are used intensively for growing flower bulbs. The topsoil is low-humic dune sand and the sandy subsoil contains shell fragments. The groundwater table was at a depth of 0·8 m.

All subsoils were collected from a layer below the groundwater table. First, plastic casings (inner diameter 75 mm) were pushed down and cleared out step by step to the sampling depth. At the first four locations, the subsoil material was collected with a stainless steel tube (length 0·5 m; inner diameter 60 mm), while retaining the wet material by applying power-driven vacuum at the closed upper end.[12] The material from two to six boreholes per field was put in polypropene barrels (content 14 or 25 dm^3), which were filled up with groundwater and closed air-tight with a polypropene cap. Between the collection of the subsoils and the start of the transformation experiment (about three weeks), they were stored in the dark at 10°C.

In Wassenaar, the subsoil material was collected *via* the casings with a stainless steel tube (length 0·4 m; inner diameter 66 mm), connected to low-pressure vessels and a hand-driven suction pump.[13] The material from three boreholes in each of the two fields was put in large flasks (2·5 dm^3), which were filled up with groundwater and closed air-tight. This material was stored for 1·5 years in the dark at 10°C.

The composition of the subsoils was measured by the Laboratory for Soil and Crop Testing in Oosterbeek, the Netherlands (Table 1). The textural class of the materials was sand and the organic matter contents were low. The Genderen and Wassenaar subsoils contained calcium carbonate in the form of shell remnants.

The redox potential was measured in two to four boreholes in each field using the method described elsewhere.[13] Values were read 15 to 60 min after insertion of the Pt electrode into the soil. The redox potential measured was corrected for the difference in potential between the Ag/AgCl reference electrode and the

TABLE 1
Collection Depth and Composition of the Subsoils

Location	*Depth (m)*	*Content (%)*			
		Clay (0–2 μm)	*Silt (2–50 μm)*	*Organic matter*	$CaCO_3$
Borgerswold	2·0–3·0	3·4	7·1	0·0	0·0
Papenvoort	2·5–3·5	2·2	5·4	0·1	0·0
Vierlingsbeek	2·5–3·5	3·1	3·7	0·0	0·1
Genderen-1988	1·8–2·7	4·9	5·5	0·5	1·5
Genderen-1989	1·8–2·7	5·6	6·4	1·0	1·6
Wassenaar-1	1·5–2·5	2·8	0·7	0·0	3·2
Wassenaar-2	1·5–2·5	1·9	1·0	0·0	5·9

normal hydrogen electrode. The pH in the subsoils was measured in the barrels transported to the laboratory, using a combination glass electrode connected to a VarilapH 1B pH/mV meter, calibrated with standard buffer solutions of pH 4 and pH 7.

2.2 Incubation

The subsoil materials from Borgerswold, Papenvoort, Vierlingsbeek and Genderen-1988 were incubated in glass flasks (250 cm^3) with screw caps containing a Teflon-coated rubber inlay. The subsoil was transferred from the collection barrels to the flasks by using a small cylinder, as described previously.[12] Meanwhile, the headspace in the collection barrel was flushed with nitrogen gas. At regular intervals, some subsoil was transferred to an aluminium tray for a determination of the water content. The glass flasks were flushed with approximately 0·5 dm^3 nitrogen gas during filling. A volume of 10 cm^3 groundwater (collected at the same site as the subsoil material) was added to each flask, ensuring the presence of a water layer (about 0·5 cm) on top of the layer of water-saturated subsoil material (about 2 cm). After the addition of the groundwater, each flask was flushed twice with 1 dm^3 of nitrogen gas, closed and stored at 10°C in the dark for one or two days. The average masses of solid phase in the systems were 90, 100, 94 and 86 g, respectively, for the four subsoil materials. The average masses of liquid phase were 38, 34, 33 and 37 g, respectively.

The incubation started by adding 0·50 cm^3 of an aqueous solution with analytical-grade atrazine or analytical-grade bentazone (both 5·0 mg dm^{-3}). The initial contents of atrazine and bentazone were about 0·02 mg kg^{-1} (on wet soil basis). The initial concentrations in the water layer were approximately 0·07 mg dm^{-3}. The flasks with the materials from Borgerswold, Papenvoort and Genderen-1988 were then flushed with 1 dm^3 nitrogen gas and their caps were closed tightly. The flasks with the Vierlingsbeek material were covered loosely, in view of the aerobic condition of this subsoil in the field. All flasks were gently swung round to obtain good mixing of the pesticide through the subsoil materials and they were incubated in a temperature cabinet in the dark at 10($\pm$1)°C.

The subsoil material collected at Genderen in 1989 was incubated in glass serum flasks (250 cm^3) with aluminium screw caps containing a butyl-rubber inlay. The 60 flasks contained an average of 84 g soil (dry mass) and 41 g water. The serum flasks were randomly divided into two equal groups. One group was stored for two days at 10°C in the dark, while the other group was sterilized by γ-irradiation (25 kGy) at Gammaster (Ede, The Netherlands). A volume of 2 cm^3 of an aqueous solution containing analytical-grade atrazine (2·5 mg dm^{-3}) was added to all flasks with the Genderen-1989 material *via* injection through the butyl-rubber inlay. During the injection, the aqueous solution was filtered through a sterile filter with pores of 0·45 μm. Before injection, the inlay was sterilized on the outside with a tissue wetted with distilled methanol. Between injections, the needle was sterilized in a gas flame. The initial content of atrazine in the wet soil was about 0·04 mg kg^{-1} and the initial concentration in the water 0·12 mg dm^{-3}.

Atrazine was incubated in the Wassenaar-1 subsoil in glass round-bottom flasks (500 cm^3) with a ground-glass stopper. Wet subsoil material (200 g) was weighed into the flasks and 20 to 25 cm^3 groundwater was added. Bentazone was incubated in the Wassenaar-2 subsoil in glass wide-neck flasks (250 cm^3) with screw caps plus polyethene inlay. Wet subsoil material (55 g) was weighed into the flasks and 5 cm^3 groundwater was added. In both incubation systems this resulted in a layer of subsoil material of about 3 cm, covered by a water layer of 0·5 cm. The headspace of the flasks was flushed with 0·5 dm^3 of nitrogen gas and this was repeated on the next two days. Then 0·50 cm^3 of an aqueous solution containing analytical-grade atrazine (10·0 mg dm^{-3}) or analytical-grade bentazone (2·5 mg dm^{-3}) was added, leading to herbicide contents of approximately 0·02 mg kg^{-1} (on wet soil basis). The

flasks were closed with ground-glass stoppers, which were kept wet during the whole incubation study to prevent losses from the flasks. They were incubated in a temperature cabinet in the dark at 10(±1)°C.

Prior to extraction, the redox potential in the subsoil in most flasks was measured with a Pt electrode and an Ag/AgCl reference electrode. Both electrodes were inserted into the subsoil material and the redox potential was read after a stabilization time of 15 to 30 min. The pH was measured using a digital pH meter (Philips PW 9408) equipped with a combined calomel electrode. The pH meter was calibrated using buffers (pH 4 and pH 7). The electrode was placed in the subsoil layer and the pH was read after 1 min.

2.3 Extraction and analysis

2.3.1 Atrazine

Two methods were used for the extraction and analysis of atrazine. The first method consisted of extraction with methanol and analysis *via* high pressure liquid chromatography (HPLC). The second method consisted of extraction with dichloromethane and analysis *via* gas liquid chromatography (GLC). The methanol/HPLC method was used for the Wassenaar-1 subsoil material up to 147 days of incubation and for the Borgerswold, Papenvoort, Vierlingsbeek and Genderen-1988 subsoil materials up to 32 days of incubation. The dichloromethane/GLC method was used in all other cases. Usually, at least three incubation systems of each subsoil material were extracted at each measuring time.

In the first method, the Wassenaar-1 subsoil was extracted with 200 cm^3 methanol and the other subsoils with 100 cm^3 methanol, by shaking for 1 h. Subsamples (10 cm^3) of the extracts were mixed with aqueous acetic acid (1 g dm^{-3}; 25 cm^3). This mixture was transferred to 150 mg adsorption material (Baker Chem Co.), with benzene sulfonic acid ($C_6H_5SO_3H$) as the bound phase, in a syringe. The adsorption material had been conditioned with 5 cm^3 acetic acid solution. After application of the subsample, the adsorption material was washed with acetic acid solution (1 cm^3), distilled water (5 cm^3) and aqueous KH_2PO_4 (0·1 M; 1 cm^3). Atrazine was eluted with acetonitrile + 0·2 M KH_2PO_4 (1 + 1 by volume). The atrazine concentrations were measured by HPLC. The analytical column was a Chrompack CP-tm-Spher C_{18} column (length 20 cm; ID 0·3 cm). The mobile phase was acetonitrile + water (45 + 55 by volume) at a flow rate of 1 cm^3 min^{-1}. Atrazine was detected with a UV-spectrophotometer at 223 nm; its retention time was 3·5 min. The detection limit corresponded to less than 4% of the amount of atrazine dosed to the incubation systems. The extraction efficiency was found to be 101(±4)% and 104(±1)% at atrazine contents of 0·05 and 0·01 mg kg^{-1}, respectively.

In the second method, atrazine was extracted by adding aqueous calcium chloride solution (0·01 M; 25 cm^3) and dichloromethane (50 cm^3). The flasks were shaken for 5 h. After storing overnight at −18°C and after thawing at room temperature, the dichloromethane and water layers were separated further *via* centrifugation. A volume of 10 to 40 cm^3 dichloromethane was collected and evaporated with a rotating film evaporator. The residue was dissolved in 2 to 5 cm^3 acetone and this solution was analysed by GLC. The gas chromatograph (Hewlett–Packard HP 5890) was equipped with a Chrompack WCOT fused-silica column (length 25 m; ID 0·53 mm), coated with a film of CP-Sil 5 (5 μm). The carrier gas was nitrogen, at a flow rate of 10 cm^3 min^{-1}. Atrazine was detected by means of an NP-detector and the detector gas was hydrogen + medicinal air + nitrogen (3 + 77 + 20 by volume), at a flow rate of 116 cm^3 min^{-1}. The temperatures of the injector, column and detector were 240, 200 and 300°C, respectively. The retention time of atrazine was 4·0 min. The detection limit corresponded to less than 2% of the amount of atrazine dosed to the incubation systems. The mean extraction efficiency at an atrazine content of 0·12 mg kg^{-1} (tested in triplicate for the Borgerswold, Papenvoort and Genderen-1988 materials) was 97% ($n = 9$; $s = 4\%$).

After 5·3 years of incubation, hydroxy-atrazine in the Borgerswold, Papenvoort and Vierlingsbeek subsoils was analysed by Ciba-Geigy (Lütolf, W., 1994. Ciba internal method. Ciba-Geigy Ltd, Basle, Switzerland). For each subsoil, an incubation system with bentazone was used as a blank for atrazine. The incubation systems with subsoil were frozen at −25°C prior to their transport to Switzerland, and then stored for eight months at −25°C. After drying 20 g of subsoil, hydroxy-atrazine was extracted by refluxing with methanol + water (1 + 3 by volume; 200 cm^3) for 1 h. Hydrochloric acid (0·25 M; 1 cm^3) was added, followed by shaking and centrifugation for 10 min at 50 rev sec^{-1}. The liquid phase was extracted twice with dichloromethane + hexane (4 + 1 by volume). The organic phase was discarded. The water phase was returned to the soil residue and concentrated hydrochloric acid (37%) + methanol (1 + 24 by volume; 100 cm^3) were added. After refluxing for 1·5 h and filtering, 100 cm^3 of the liquid phase was evaporated to 15 cm^3 at 40°C under reduced pressure. Hydrochloric acid (0·24 M; 5 cm^3) was added and the liquid phase was cleaned using 3 cm^3 swollen cationic exchange resin (AG50W-X8, H^+-form) conditioned with water until the pH of the eluate was 7. After application of the refluxed liquid phase, the resin was washed with methanol (2 × 25 cm^3) and then with 25% aqueous ammonia + methanol (5 + 95 by volume; 5 cm^3). Hydroxy-atrazine was eluted with 25% aqueous ammonia + methanol + water (1 + 6 + 3 by volume). The liquid was evaporated at 40°C under reduced pres-

sure and the residue was dissolved in methanol + 3% ammonium acetate buffer at pH 5 (3 + 7 by volume). The recovery of hydroxy-atrazine from the Borgerswold subsoil in a single experiment was 122%. Recoveries from 'normal soil' were 94% ($n = 4$; $s = 8\%$) at a content of 0·04 mg kg^{-1}, 60% ($n = 3$; $s = 13\%$) at a content of 0·4 mg kg^{-1} and 83% (singular) at a content of 0·8 mg kg^{-1}.

The concentrations of hydroxy-atrazine were measured by HPLC. The analytical column was an Inertsil C8 column (length 25 cm; ID 0·46 cm) produced by VDS Optilab Chromatographie Technik. Hydroxy-atrazine was eluted with methanol + 3% ammonium acetate buffer at pH 5 (3 + 7 by volume), at a flow rate of 1·0 cm^3 min^{-1}. The retention time of hydroxy-atrazine, detected with a UV/VIS-spectrophotometer at 225 nm, was 20 min. At the injection volume of 50 mm^3, the detection limit was 0·01 mg kg^{-1} (based on wet subsoil). This detection limit corresponded to 55% of the initial atrazine content.

2.3.2 Bentazone

Two extraction methods were used for the analysis of bentazone. In the first method the ethyl acetate extracts were subjected to a clean-up procedure. In the second method the water phase was extracted with dichloromethane and there was no clean-up. The first method was used for the Wassenaar-2 subsoil up to 174 days of incubation and for the Borgerswold, Papenvoort, Vierlingsbeek and Genderen-1988 subsoils up to 32 days of incubation. The second method was used in all other cases. Usually, at least three incubation systems of each subsoil were extracted.

In the first method bentazone was extracted by adding calcium chloride solution (100 cm^3) to the subsoil and shaking for 1 h. After centrifugation for 10 min, 40 cm^3 of the water layer was combined with hydrochloric acid (0·25 M; 25 cm^3) and ethyl acetate (50 cm^3), followed by shaking for 1 h. The ethyl acetate layer was dried with anhydrous sodium sulfate, after which 30 cm^3 of the ethyl acetate layer was evaporated using a rotary evaporator at 40°C. The residue was dissolved in sodium phosphate buffer (0·05 M; pH 8; 4 cm^3) and dichloromethane (4 cm^3) was added for clean-up. After shaking and centrifugation, the dichloromethane was discarded. Freshly prepared tetrabutylammonium hydrogen sulfate (0·01 M; 0·2 cm^3) in sodium phosphate buffer (0·05 M; pH 8) was added to 3 cm^3 of the aqueous layer. Dichloromethane (3 cm^3) was then used to extract the bentazone-tetrabutylammonium complex by shaking for 2 min, followed by centrifugation. Two cubic centimetres of the dichloromethane layer was evaporated in a waterbath at 40°C with an air stream. The residue was dissolved in aqueous acetic acid (4×10^{-5} M) + methanol (9 + 1 by volume; 1 cm^3). The bentazone concentrations were measured by HPLC. The analytical column was a Chrompack CP-tm-Spher C_{18} column (length 20 cm; ID 0·3 cm) in an oven at 30°C. The mobile phase was aqueous acetic acid (4×10^{-5} M) + methanol (9 + 1 by volume), at a flow rate of 1·0 cm^3 min^{-1}. Bentazone was detected with a UV-spectrophotometer at 224 nm; its retention time was 4·5 min. The detection limit corresponded to less than 2% of the amount of bentazone dosed to the incubation systems. Two experiments in triplicate, using contents of 0·02 mg kg^{-1}, showed the extraction efficiency of this method to be 98% ($s = 3$ and 5%, respectively).

In the second method, bentazone was extracted by shaking the subsoil with aqueous calcium chloride solution (0·01 M; 50 cm^3) for 1 h. After the soil had settled, 40–70 cm^3 of the water layer was collected and hydrochloric acid (0·25 M; 25 cm^3) plus 25–60 cm^3 dichloromethane were added. This was followed by shaking for 1 h and centrifugation. The dichloromethane layer (20–50 cm^3) was evaporated with a rotary evaporator at 40°C. The residue was dissolved in aqueous sodium acetate (0·1 M; pH 5·0) + methanol (85 + 15 by volume). The bentazone concentrations were measured by HPLC. The mobile phase was sodium acetate buffer (0·1 M; pH 5·0) + methanol (85 + 15 by volume) at a flow rate of 0·8 cm^3 min^{-1}. Bentazone was detected with a UV-spectrophotometer at 224 nm; its retention time was 11·2 min. The detection limit corresponded to 0·25% of the bentazone dosed to the incubation systems. The extraction efficiency was measured in three experiments with different subsoils (two in duplicate and one in triplicate) at a content of 0·08 mg kg^{-1}; the average value was 93% ($n = 7$; $s = 9\%$).

3 RESULTS

3.1 pH and redox potential

The pH values measured in the collection barrels and in the incubation systems are presented in Table 2. The values for the barrels covered a rather wide range, from pH 4·5 (Papenvoort and Vierlingsbeek subsoils) to around 7·6 (Wassenaar subsoil). The pH values measured in the incubation systems were close to those in the collection barrels.

The redox potentials measured in the boreholes in the field are also shown in Table 2. The comparatively high value for Vierlingsbeek indicates that this subsoil was in an aerobic condition. The two Genderen subsoils were in a rather strongly reduced condition. The measurements for different boreholes in the same field indicate that the variation in redox potential can be substantial.

Over the first year, the redox potential in the incubations systems with the Borgerswold and Papenvoort subsoils (Table 2) increased somewhat (compared to the

TABLE 2
pH Values of the Subsoils Measured in the Collection Barrels and in the Incubation Systems and Redox Potentials of the Subsoils Measured in the Boreholes in the Field and in the Incubation Systems

	pH in		*Redox potential (V) in*	
Location	*Barrel*	*Incubation system*[a]	*Borehole*	*Incubation system*[a]
Borgerswold	5·7	5·2 (4·8–5·7)	0·49	0·57 (0·43–0·65)
Papenvoort	4·5	4·3 (4·1–4·5)	0·36–0·50	0·64 (0·48–0·69)
Vierlingsbeek	4·5	4·5 (4·2–4·8)	0·71	0·65 (0·56–0·69)
Genderen-1988	7·0	6·8 (6·3–7·2)	0·07–0·10	0·16 (−0·06–0·26)
Genderen-1989		7·2 (6·8–7·6)	0·07–0·11	0·08 (−0·03–0·16)
Wassenaar-1	7·4–7·7	7·7 (7·2–8·3)	0·21–0·35	0·41 (0·27–0·48)
Wassenaar-2	7·4–7·7	7·6 (7·1–7·9)	0·21–0·35	0·37 (0·27–0·47)

[a] Average values followed by range in parentheses.

values found in the boreholes). They remained at the same elevated level over the subsequent four years. During the incubation period, the redox potential of the Vierlingsbeek subsoil was somewhat lower than that in the field.

The average redox potential in 15 of the 21 incubation systems with the Genderen-1988 subsoil was slightly higher than that in the field, while the range was wider (Table 2). However, the other six incubation systems showed a redox potential higher than 0·4 V. In four of these systems the colour of the subsoil had changed from blue/grey to brown, indicating that the subsoil had passed into an oxidized state. Presumably, some air had leaked along some of the septa. During the incubation with the Genderen-1989 subsoil, the redox potential remained at the same comparatively low level as that measured in the boreholes in the field.

The redox potential for the incubations with the Wassenaar subsoil was on average about 0·1 V higher than that in the field (Table 2). There was some fluctuation in redox potential with time of incubation, with highest values around 0·4 V after two years and lowest values around 0·3 V after four years.

3.2 Transformation of atrazine

The results of the incubation of atrazine in the Borgerswold subsoil (Fig. 1) show that the amount of the herbicide decreased over time. After 5·3 years of incubation, 1·9% of the dose ($n = 3$; $s = 0{\cdot}2\%$) was left. The course of the transformation could be described reasonably well by first-order kinetics. The corresponding half-life was 0·91 year (coefficient of variation $cv = 5\%$). Shortly after the start of the experiment, the differences between the amounts of atrazine in the individual incubation systems were small. In the course of time, these differences tended to increase as the amount of atrazine slowly decreased. The differences became smaller again after more than 90% of the initial amount of atrazine had been transformed.

Atrazine incubated in the Papenvoort subsoil was transformed gradually (Fig. 1), until 6·2% of the dose ($n = 3$; $s = 0{\cdot}5\%$) was left after 5·3 years. Again, the course of the transformation could be described reasonably well by first-order kinetics. The corresponding half-life was 1·5 years ($cv = 8\%$). The differences between the amounts of atrazine in the individual incubation systems were small, except for two times after about one year of incubation.

In the incubation systems with Vierlingsbeek subsoil, there was no distinct transformation of atrazine in the first 1·6 years (Fig. 1). However, after 3·9 and 5·3 years of incubation, the amount of atrazine in all systems had clearly decreased. At the end of the incubation period, 17·5% of the dose ($n = 3$, $s = 3{\cdot}2\%$) was left. The course of the transformation between 1·6 and 5·3 years could be described reasonably well by first-order kinetics. The corresponding half-life was 1·6 years ($cv = 13\%$).

Atrazine in the Genderen-1988 subsoil material was transformed at a comparatively high rate (Fig. 1). A half-life of 0·26 year ($cv = 10\%$) could be calculated, assuming first-order kinetics. Because of this comparatively fast transformation, the experiment was repeated with subsoil collected at Genderen in 1989. The transformation of atrazine in the latter material (Fig. 1) was found to be even more rapid than in the subsoil material collected in 1988. Again, the transformation could be described by first-order kinetics; the corresponding half-life of atrazine in Genderen-1989 subsoil was 0·16 year ($cv = 15\%$).

The rate of transformation of atrazine in the sterilized Genderen-1989 subsoil material (Fig. 1) was not lower than that in the corresponding non-sterilized subsoil. It was also described by first-order kinetics; the corresponding half-life of atrazine was 0·14 year ($cv = 7\%$).

The amount of atrazine in the systems with Wassenaar-1 subsoil hardly decreased over a period of

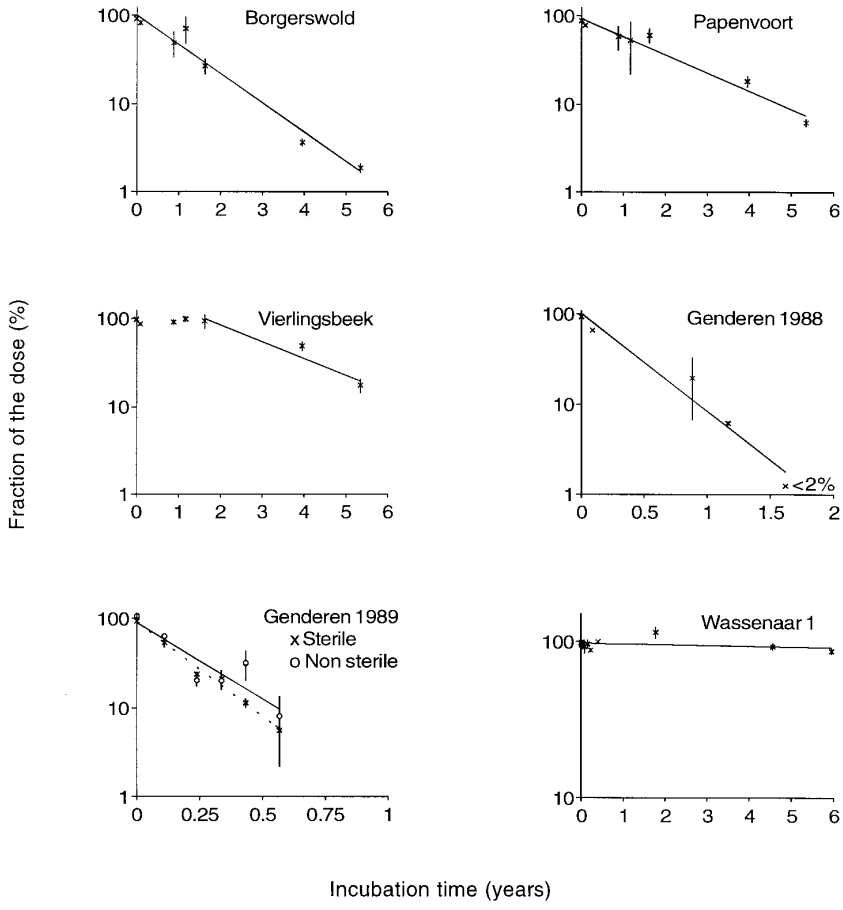

Fig. 1. Rate of transformation of atrazine in subsoils from Borgerswold, Papenvoort, Vierlingsbeek, Genderen-1988, Genderen-1989 and Wassenaar-1. Average percentage of atrazine measured, with standard error. Lines: approximation by first-order kinetics. Note the differences in time scale.

six years (Fig. 1). After 4·6 years, 92% ($n = 3$; $s = 4\%$) of the dose remained and after six years 87% ($n = 3$; $s = 4\%$). In view of the slow transformation of atrazine, accurate calculation of the transformation rate and half-life was not possible.

After 5·3 years of atrazine incubation with Borgerswold subsoil, hydroxy-atrazine was detected in duplicate systems at a content of 0·01 mg kg^{-1}. The incubation of atrazine with the Papenvoort and Vierlingsbeek subsoils did not yield hydroxy-atrazine (detection limit 0·01 mg kg^{-1}). When no atrazine was dosed to the three subsoils (blank systems), the hydroxy-atrazine content was always lower than 0·01 mg kg^{-1}, as expected. It should be noted that 0·01 mg of hydroxy-atrazine per kg corresponded to 55% of the dose of atrazine. Any lower percentages of hydroxy-atrazine formed were below the detection limit.

3.3 Transformation of bentazone

Over the first 1·6 years of the incubation of bentazone with the Borgerswold subsoil, there was a gradual transformation to 74% ($n = 6$; $s = 7\%$) of the dose (Fig. 2). Only 3% of the dose remained after 3·9 years. Later (after 5·0 years), bentazone could no longer be detected; the amount in the systems (five-fold) had become smaller than 0·25% of the dose. The transformation of bentazone could not be described by first-order kinetics because the transformation rate coefficient increased over time.

In the Papenvoort subsoil material, bentazone was transformed gradually until 11% of the dose ($n = 5$; $s = 6\%$) remained after 5·0 years (Fig. 2). As indicated by the line, the course of the transformation could be approximated by first-order kinetics; the corresponding half-life was 1·5 years ($cv = 9\%$). The differences between the incubation systems tended to increase as the transformation of bentazone proceeded.

In the Vierlingsbeek subsoil material, there was a gradual transformation of bentazone to leave 25% ($n = 5$; $s = 11\%$) of the dose after 5·0 years (Fig. 2). The course of the transformation was described by first-order kinetics; the corresponding half-life was 2·5 years ($cv = 8\%$). At 5·0 years, the differences between individual incubation systems were greater than at the earlier times.

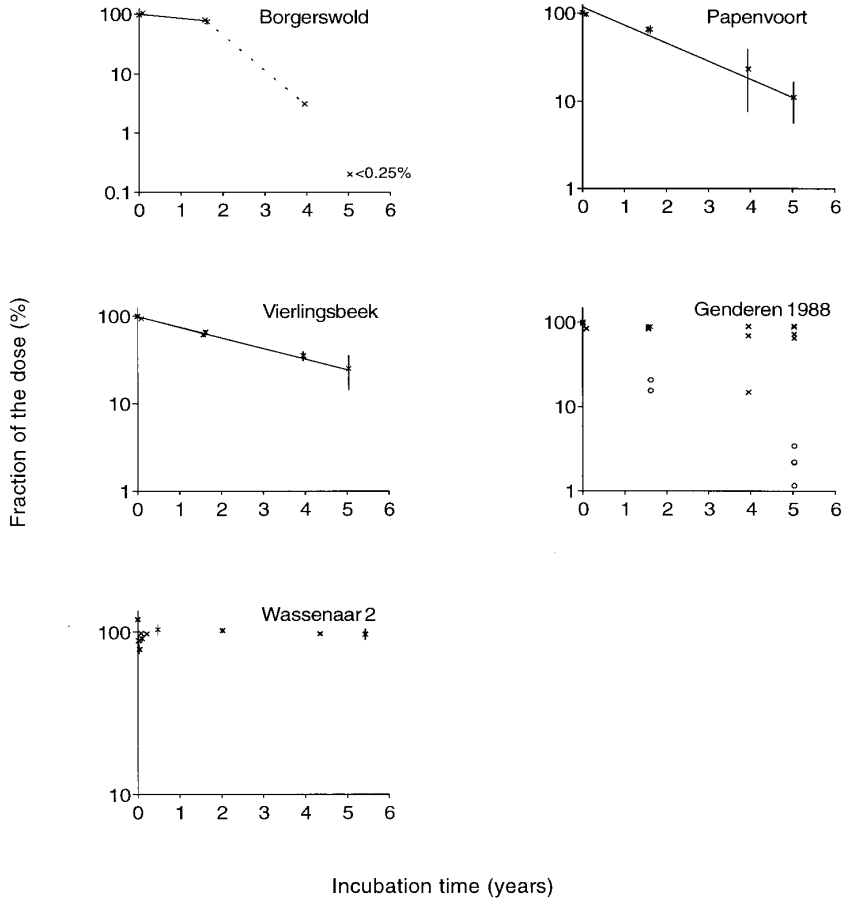

Fig. 2. Rate of transformation of bentazone in subsoils from Borgerswold, Papenvoort, Vierlingsbeek, Genderen-1988 and Wassenaar-2. Average percentage of bentazone measured, with standard error. Lines represent approximations by first-order kinetics. Percentage of bentazone in individual incubation systems with a redox potential (×) lower or (○) higher than 0·26 V (Genderen-1988). Note the differences in the scales of the vertical axes.

The results for the incubation of bentazone with Genderen-1988 subsoil are shown in Fig. 2. The percentages remaining over the years show a wide degree of scatter. A distinction was made between the incubation systems in which the redox potential remained comparatively low (as it had been in the field) and those in which the redox potential had increased substantially due to leakage of air (Section 3.1). Bentazone transformation in the systems with comparatively low redox potential was very slow. There was one exception to this general tendency: in one system, 14% of bentazone remained after four years of incubation, though the redox potential in the subsoil was −0·06 V. In the systems with comparatively high redox potential, bentazone was transformed at a substantial rate (Fig. 2).

The amount of bentazone in the systems with Wassenaar-2 subsoil hardly changed in 5·4 years (Fig. 2): an average of 96% ($n = 3$; $s = 8\%$) of the dose could be measured at the end of the incubation. The transformation of bentazone was too slow to allow calculation of its half-life.

4 DISCUSSION AND CONCLUSIONS

Most of the incubations of atrazine in the present study were continued for several years, because its rate of transformation in the water-saturated subsoil materials was low, making it difficult to derive a rate of transformation from the measurements in the first year of incubation. In some published studies, the time of incubation of atrazine in aquifer material was comparatively short. It has been reported, for instance, that after incubation in aerobic aquifer material (pH 7·2 to 7·8) at room temperature for 74 days, no transformation of atrazine could be detected.[14] In another study, atrazine was incubated in aquifer material (aerobic; pH 5·5) at 10°C for 174 days.[15] Again the rate of transformation over that period was too low to be measured. In various aquifer materials, atrazine has to be incubated for several years to allow measurement of its rate of transformation.

The Borgerswold, Papenvoort and Vierlingsbeek subsoils had comparatively low pH values and their redox

potential was moderate to high. The subsoils from Genderen and Wassenaar had the highest pH values and their redox potential was comparatively low. Surprisingly, the rates of transformation of atrazine in the latter two subsoils were very different. After sterilization of the Genderen-1989 subsoil material, the transformation rate remained high, which indicates that transformation was mainly a chemical process. The organic matter content of the Genderen subsoils was distinctly higher than that of the Wassenaar-1 subsoil. The transformation of atrazine may have been catalysed by this constituent. Another possibility is that reductive dechlorination of atrazine occurred at the low redox potential in the Genderen subsoils, whereas it did not occur at the higher redox potential in the Wassenaar subsoil.

The detection of hydroxy-atrazine in the Borgerswold subsoil material indicates that it can be an important transformation product in aquifers. The detection limit should be lowered to measure smaller fractions of the atrazine dose present as hydroxy-atrazine and to follow its concentration in time.

There was a gradual transformation of bentazone in the Borgerswold, Papenvoort and Vierlingsbeek subsoils, at comparatively low pH values and moderate to high redox potentials. The rates of bentazone transformation in the Genderen-1988 and Wassenaar-2 subsoils, with comparatively high pH and in rather strongly reduced conditions, was low. However, after oxygen had accidentally entered some incubation systems with Genderen-1988 subsoil, the transformation of bentazone became much faster. The comparatively high pH of the latter subsoil was not an obstacle to this higher transformation rate.

No results of studies on the transformation of bentazone in aquifer materials could be found in the literature. The rate of hydrolysis of bentazone in water (pH range 5 to 9) under laboratory conditions is very low.[16] This explains why bentazone was only slowly transformed in some subsoils. In a general characterization of the behaviour of bentazone in soils, it was reported that its transformation practically ceases under anaerobic conditions.[16] The slow transformation in the two subsoils with the lowest redox potential in the present study was in agreement with these findings.

Protracted incubation of pesticides in water-saturated subsoil materials seems to be possible in the case of abiotic transformation. However, the microbial activity in the subsoils may decrease over time. The storage time of 1·5 years of the Wassenaar subsoils may have slowed down microbial transformation. However, the transformation of bentazone in the first three subsoils continued for several years and this may be a microbial process. It is not known yet how long incubation in water-saturated subsoils can be continued if transformation mainly depends on microbial activity.

This study shows that the rate of transformation of a pesticide in various subsoils can vary greatly. Information on the factors affecting this rate is scarce. Pesticides that may leach to the upper groundwater should thus be incubated in the most relevant subsoils of the regions in which they are applied. The conditions in the aquifer have to be simulated as adequately as possible in the incubations. Furthermore, the formation of transformation products requires more attention.

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